

ABSTRACT

Charles University in Prague

Faculty of Pharmacy in Hradec Králové

Department of Biochemical Sciences

Candidate: Lucie Voborová

Supervisor: PharmDr. Petra Malátková, Ph.D.

Title of diploma thesis: Construction of promoter-luciferase constructs for DHRS3 gene

Membrane-bound reductases, which include DHRS3, are still under-researched area. It is assumed that DHRS3 plays a role in the metabolism of retinoids, which contributes to maintaining the balance of all-*trans*-retinoic acid (ATRA) signaling and thereby regulates the formation of body axis. Gene regulation of DHRS3 by retinoids has not been described yet. Therefore, we decided to examine the gene regulation of DHRS3. The clarification of gene regulation may tell more about DHRS3 function. In order to perform the analysis of the promoter it was necessary to prepare luciferase constructs containing varying lengths of the promoter region of DHRS3. The preparation of the constructs involved amplification, restriction, ligation, transformation, colony PCR, restriction analysis and sequencing. The individual steps were optimized for specific constructs so as to achieve their correct assembly. Thanks to appropriate procedures and optimization constructs were created in sufficient quantity and quality, and can thus be used in the analysis of the promoter using luciferase assay.

Key words:

Cloning, dehydrogenase/reductase, DHRS3, gene regulation, all-*trans*-retinoic acid